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Effect Of Space Flight on Adrenal Medullary Function

NAG2-1201

Final report (through 8/31/1999)

By

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OCT 20 2000
U: 202A-3
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Abstract: We hypothesize that microgravity conditions during space flight alter the expression and specific activities of the adrenal medullary CA synthesizing enzymes (CASE). Previously, we examined adrenals from six rats flown for six days aboard STS 54 and reported that microgravity induced a decrease in the expression and specific activity of rat adrenal medullary tyrosine hydroxylase, the rate limiting enzyme of CA synthesis, without affecting the expression of other CASE. In the past, we analyzed some of the > 300 adrenals from two previous Space Shuttle missions (PARE 03 and SLS 2). The preliminary results (a) attest to the good state of tissue preservation, thus proving the feasibility of subsequent large-scale evaluation, and (b) confirm and extend our previous findings. With this grant we will be able to expeditiously analyze all our specimens and to complete our studies in a timely fashion. As in the original proposal, the scientific aims of this study are:

- (1) To determine the effects of space flight on the total adrenal medullary catecholamine content by using reversed phase HPLC with electrochemical detection.
- (2) To measure the specific activities of three of the major catecholamine synthesizing enzymes, tyrosine hydroxylase, dopamine-hydroxylase, and phenylethanolamine-N-methyltransferase using established radioenzymatic/colorimetric assays.
- (3) To assess the amount of immunoreactive catecholamine synthesizing enzymes by quantitative Western blotting.
- (4) To evaluate the effects of space flight on the differential gene expression of catecholamine synthesizing enzymes by quantitative RT-PCR.

Given the wealth and value of the specimens at hand, their thorough analysis will be important for assessing the adaptation of astronauts to microgravity in space and to one-g conditions upon return to earth.

Task Progress: This "final report", covering the period until August 31, 1999, was necessitated by having to close down the laboratories at the Milwaukee Clinical Campus and moving to the main campus of the University of Wisconsin in Madison. As such, this "final report" actually represents a report on work in progress. Our work at the Milwaukee Clinical has mainly focused on the most time consuming of all tasks, namely the analysis of tissue catecholamine contents by HPLC, as described in specific aim 1. We have also made progress in the quantization of

immunoreactive catecholamine synthesizing enzyme protein expression in the tissues, as per specific aim 3. For this, we have used approximately 1/2 of our samples from PARE.03 and SLS-2 and all our limited samples from Neurolab. So far we have performed more than 600 HPLC analyses. At the time of dismantling the laboratory in Milwaukee, we just had synthesized the necessary molecular biological tools (probes) for assessing the expression of the genes for the catecholamine synthesizing enzymes, using RNase protection assays.

The results from the HPLC studies of PARE.03 and SLS-2, but, interestingly, not from Neurolab, seem to support our previous findings from STS-54, that exposure to space flight leads to a depression in tissue catecholamines. Furthermore, using the large sample size, we now find, that the ratio of epinephrine/norepinephrine in the tissues is decreased, which may be due to a reduction in the production of epinephrine. Also, as another novel finding, we observed that this effect was long lasting (for up to 24 hours post re-entry). In the past, we had only samples that were dissected < 6 hours upon re-entry.

By contrast, in hind limb suspended controls (as a way to "simulate" microgravity), tissue catecholamines were significantly increased. The availability of this "simulated microgravity" control, which, from a hormonal point of view, is more suited as a stress model, helps to clearly discern a space-flight/reentry associated reduction of tissue catecholamines, from the "stress" response.

The immunological analysis of the CASE proteins by Western blotting is ongoing and too preliminary, as to draw solid conclusions. However, it appears that the reduced level of epinephrine production in the post-reentry flight samples is paralleled by a similar reduction in the key enzyme, phenylethanolamine-N-methyl transferase (PNMT).

Future plans: With the re-establishment of our laboratory in Madison, we plan on proceeding according to our original plans: The samples used for HPLC and Western blots, will also be used for the enzymatic analysis of the CASE proteins (specific aim 2), which is the main task for the remaining year. The remainder of the samples will be used for assessing the expression of the CASE steady state mRNA by RNase protection assay, rather than by quantitative RT-PCR. In the past year we have gained more experience with this technique, which appears to be less prone to pitfall than qRT-PCR. We anticipate that we will finish the study in a timely manner, as originally proposed, and that we then can prepare the results for presentations at scientific meetings and for publication in peer-reviewed journals

Conclusions: Our results seem to indicate a significant difference in terms of adrenal catecholamine contents between tissues from animals exposed to microgravity in space and those obtained from tail-suspended animals. The results from the latter group indicate that tail-suspension, a frequently used model for "microgravity," generated sufficient "stress" to affect the hormonal balance in the test animals. Hence the outcome of these studies should take into account possible hormonal/stress effects in ascribing endocrine response to exposure to (simulated) microgravity.

Significance: The work performed at The Milwaukee Clinical Campus appears to further substantiate our previous findings, that space flight alters the level and ratio of tissue catecholamines, by specifically decreasing epinephrine. These resulted, if substantiated at the enzymatic and gene level, are exciting from the basic-science point of view, since they suggest the existence of distinct microgravity-dependent mechanisms for the regulation of the various CASE. Our findings seem to support a recent theory that gravitational alterations in circulating catecholamines levels might, in part, be responsible for physiological consequence of weightlessness and cardiovascular abnormalities upon return from space. Thus, our findings are consequential for space flight in general and might provide new pharmacological approaches for the management of physiological imbalances during space flight and upon re-adaptation to 1 g.